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MANUFACTURE OF SIX LOTS OF
STAPHYLOCOCCUS ENTEROTOXOID B

FINAL REPORT

by

D. S. Mabry, Ph.D.

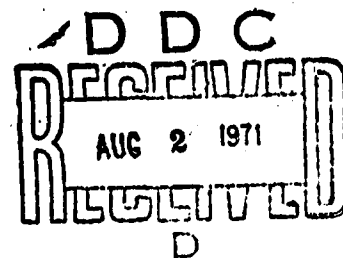
July 1971

(For the period 19 February 1968 to 28 February 1970)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

Contract No. ~~DADA~~17-68-C-8079
Pfizer Inc.
New York, New York 10017



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| 13. ABSTRACT This contract was a best effort attempt to prepare six lots of Staphylococcus Enterotoxoid B in such a manner as to be suitable for human clinical trial. The facilities, manufacturing process and test procedures were designed for submission to the Division of Biologic Standards of the National Institutes of Health as an Investigational New Drug. The United States Army Medical Research and Development Command supplied purified Staphylococcus Enterotoxin B. They further supplied an outline of a formaldehyde detoxification process and procedures for monkey toxicity and safety tests. Other routine tests normally required by the Division of Biologic Standards for various vaccine preparations were conducted in accordance with standard procedures. Processing and testing were conducted in Pfizer's licensed facilities for the manufacture of human vaccines (U.S. Government License No. 64). The attached report consists basically of a concise but detailed description of production methods and a detailed manufacturing and testing protocol for each of the six lots of toxoid. | | | |

DD FORM 1473

REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

| 14. KEY WORDS | LINK A | | LINK B | | LINK C | |
|--|--------|----|--------|----|--------|----|
| | ROLE | WT | ROLE | WT | ROLE | WT |
| <p>Staphylococcus Enterotoxoid B</p> <p>Description of Production Methods</p> <p>Manufacturing Process Summaries By Lot of Production:</p> <p>Staphylococcus Enterotoxoid B - Lot 96627</p> <p>Staphylococcus Enterotoxoid B - Lot 4237J8</p> <p>Staphylococcus Enterotoxoid B - Lot 4261J8</p> <p>Staphylococcus Enterotoxoid B - Lot 4291K8</p> <p>Staphylococcus Enterotoxoid B - Lot 4292K8</p> <p>Staphylococcus Enterotoxoid B - Lot 87285</p> | | | | | | |

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DESCRIPTION OF PRODUCTION METHODS -
STAPHYLOCOCCUS ENTEROTOXOID B

I. Receipt and Storage of Toxin

- A. When received, stored toxin at -20°C . or colder (3/13/68).
- B. Inventory toxin received. Toxin received lyophilized and shipped in insulated container. Vials received sealed in cans.

- 11 cans of 18 vials @ 100 mg/vial
- 5 cans of 18 vials @ 50 mg/vial
- 3 cans of 18 vials @ 10 mg/vial
- 4 cans of 18 vials @ 2 mg/vial.

II. Reconstitute

- A. Remove vials equivalent to 4 grams of toxin for a lot. Transfer in a carrier to the sterile area.
- B. Verify the toxin count and the label identification on each vial.
- C. Clean the surface of each vial with 70% alcohol after removing penetration seals.
- D. Release vacuum from each vial by inserting a 26-gauge, plugged needle through each stopper and leaving the needle in place.
- E. Using a 21-gauge needle and a 2-ml syringe or a 2-ml Cornwall syringe, add 2 ml of 0.15 M potassium phosphate buffer at pH 7.5 to each vial through the stopper. Use care to wet and resuspend all powdered toxin.
- F. Remove toxin with syringe and needle and transfer to a sterile 2-liter graduate.
- G. Repeat step II. E. and F. until after the addition of the 4th 2-ml volume.
- H. Remove the vent needle.
- I. Agitate vial to wet all surfaces.

Description of Production Methods --
Staphylococcus Enterotoxoid B

II. J. Remove aluminum seal and discard.

- K. Aseptically remove stopper and pour contents of vial into graduate.
- L. By pipette or syringe add the 5th 2-ml wash to the vial and restopper with the same stopper.
- M. Rinse all surfaces. Remove and discard stopper and add contents of vial to other washes. Discard empty vials.
- N. When all vial contents and washes have been pooled in the graduate, add additional 0.15 M buffer to bring the volume to 1,800 ml.
- O. Using a pipette add 16 ml formalin to the graduate while gently swirling the graduate.
- P. Add 0.15 M buffer to bring the volume to 2,000 ml.

III. Detoxification

- A. Transfer the contents of the 2-liter graduate into a 4-liter bottle and stopper. Cover stopper with a gauze pad and secure with rubber bands.
- B. Transfer to a water-tight receptacle along with a pilot bottle of water identical in size and contents to the toxoid bottle and containing a thermometer to be used for temperature control.
- C. Place the water-tight receptacle and its contents in a 37°C. incubator.
- D. Agitate at least daily on each working day and record temperature of pilot bottle.
- E. After 30 days detoxification, remove from the incubator to a sterile room and sample:
 - 1. Detoxification - 15 ml
 - 2. Nitrogen - 5 ml
- F. Record total days of detoxification on the label and store at 5°C.

Description of Production Methods -
Staphylococcus Enterotoxoid B

IV. Dialysis

- A. When released for dialysis, after satisfactory completion of the detoxification test, in a sterile room dialyze against 64 liters chilled, sterile 0.02 M potassium phosphate buffer at pH 7.5 at 5°C.
 1. Use 250 ml of toxoid per dialysis bag, 4 dialysis bags per stainless steel 10-gallon tank containing 32 liters of the 0.02 M buffer. Stopper dialysis bags with hooded rubber stoppers. Dialysis bags are cellulose casing size #36 purchased from Visking Corporation.
 2. Two tanks are required per lot.
 3. Be sure buffer covers above fluid levels in the dialysis bags.
 4. Place tanks at 5°C. for 48 hours.
- B. Remove tanks to a sterile room. Wipe down sides and top of tank.
- C. Aseptically pour the contents of a bag into a sterile graduate. Rinse the bag with approximately 30 ml of physiological saline and add to the graduate.
- D. Transfer the contents of the graduate to a sterile 4-liter bottle and continue emptying bags until all dialyzed fluid is contained in the 4-liter bottle.
- E. Calculate the final volume of the toxoid from the volumes recorded for the contents of each bag and its rinse.
- F. Add 1% Thimerosal solution to give a 1:10,000 concentration.
- G. Mix well and sample:
 1. Nitrogen - 5 ml
 2. Free Formalin - 8 ml
- H. Stopper the bottle, cover with a dust cap and store at 5°C.

Description of Production Methods -
Staphylococcus Enterotoxoid B

V. Millipore Filtration

- A. When it is determined that the free formalin level is less than 0.02%, remove to a sterile room for filtration.
- B. Use a sterile 293 mm Millipore filter holder containing as listed from top to bottom a prefilter, RA, AP32 257, AA, AP32 257, HA, AP32 257. Millipore AP32 257 is a dacron mesh separator.
- C. Rinse through the entire system and discard approximately 3.5 liters of physiological saline containing 1:10,000 Thimerosal.
- D. Filter the toxoid and collect in a sterile 9-liter bottle.
- E. Rinse entire system with 400 ml physiological saline containing 1:10,000 Thimerosal and add to the 9-liter bottle.
- F. Use two successive 300-ml rinses as in E. Depending on current assays these may be added to the final bottle or retained separately pending further analysis for ultimate use or discard.
- G. Mix well and sample:
 1. Nitrogen - 5 ml
- H. Bubble test the Millipore. Repeat the filtration through another filter if the bubble test shows a membrane failure.
- I. Store at 5°C. until assays are complete.

VI. Standardization

- A. On the basis of the postfiltration nitrogen assay, dilute the toxoid to a calculated final nitrogen concentration of 110 mcg/ml with physiological saline containing 1:10,000 Thimerosal.
- B. Mix well and sample:
 1. Nitrogen - 5 ml
 2. Sterility - 10 ml

Description of Production Methods -
Staphylococcus Enterotoxoid B

VI. C. Store at 5°C. until released for filling. Release for filling was based on a nitrogen assay between 100 and 120 mcg/ml and satisfactory completion of the bulk sterility test on samples taken in Step VI. B.

VII. Filling

- A. Fill into type I 5-ml serum vials and seal with 20 mm combination seals W/147 P grey liner.
- B. Use centerpoint 6.3 ml/vial with maximum 6.5 ml and minimum 6.1 ml.
- C. Sample: (Use underfills and overfills where available)
 - 1. Sterility - 20 vials
 - 2. Small animal safety - 4 vials
 - a. guinea pig
 - b. mouse
 - 3. Monkey safety - 4 vials
 - 4. Monkey potency - 3 vials
 - 5. Chemical - 4 vials
 - a. nitrogen
 - b. mercury
- D. Store at 5°C. until labeled.
- E. Dry bottles and label. Sample:
 - 1. Manufacturing File - 6 labeled vials
- F. Store at 5°C. until shipped.
- G. Ship refrigerated.

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 96627

MANUFACTURING PROCESS SUMMARY

Toxoid Lot No. 96627

Processing Started 10/16/68

In Process Lot No. 4260J8

Toxin Used - Lot 14-31R

31 x 100 mg/vial

18 x 50 mg/vial

Resuspended in 2 Liters 0.15M K phosphate buffer pH 7.5

Containing 0.8% Formalin

Date 10/16/68

Detoxification Started 37°C

Date 10/16/68

Removed to 5°C

Date 11/15/68

Time at 37°C = 30 days

Dialysis: Time 48 hours

Dialyzing Fluid - 0.02M K phosphate buffer pH 7.5

Dialyzing Fluid Volume - 64 Liters

Thimerosal Added to 1:10,000

Final Dialyzed Toxoid Volume - 2,705 ml

Filtration - Millipore 293 mm

Date 12/27/68

Prefilter

RA

AP32 257

AA

AP32 257

HA

AP32 257

Rinse Solution - Physiological Saline with 1:10,000
Thimerosal

Filtration Time - 45 minutes

MANUFACTURING PROCESS SUMMARY - Page 2.

Final Bulk Prepared

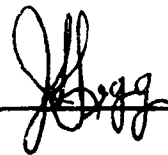
Date 6/13/69

Preservative Added - Thimerosal 1:10,000

Diluent - Physiological Saline

Final Volume After Samples - 6,380 g.

Approved by

A handwritten signature in dark ink, appearing to be 'J. H. Hagg', is written over a horizontal line.

SUMMARY OF FILLING AND LABELING OPERATION

Toxoid Lot No. 96627

Dose Size 10 Volume 5 ml Date Filled 6/17/69

Fill Volume: Max. 6.5 ml Centerpoint 6.3 ml Min. 6.1 ml
with overflow

Filling Time 2 hours Amount Filled 1,008

Packaging Materials:

Type I 5 cc Serum Vials
20 mm, 147 P Grey Combination Seals

Samples Taken: (Short Fill Vials Used)

| | | | |
|---------------------|---------|----------------|--------|
| Sterility | 20 x FC | Monkey Potency | 3 x FC |
| Small Animal Safety | 4 x FC | Chemical | 4 x FC |
| Monkey Safety | 4 x FC | | |

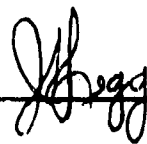
Labeled 974 vials Date Labeled 10/16/69

Additional Samples Taken:

Mgf. File 6

Finished Goods for Shipment 968

Approved by



Effective: Nov. 1967

Approved: [Signature]FILL ORDERProduct Staphylococcus Enterotoxin B Final Container No. 96627Dose _____ Size 5 cc Code _____ Max. 6.5 Cp. 6.3 Min. 6.1 Spg. _____Expiration Date _____ Date Filled 6-17-69 Amount Filled 1008 Total Fill Time 2hArea and Equipment Inspected and Satisfactory For Use Dan H. Williams Date 6-17-69

| IBM No. | Packaging Codes and Descriptions | Amount Used |
|---------|----------------------------------|---------------------|
| 30-0003 | Type I 5ml Serum Vial | 1080 ¹⁰⁵ |
| 42-0014 | 20mm 142 Pk Box Comb. Seal | 1080 ¹⁰⁵ |

| Sample No. | Type of Test | Volume | Date | By | Results | Date | Op. |
|------------|-----------------------|--------|---------|----|------------------------------------|---------|-----|
| / | STERILITY (SHORT RUN) | 20 | 6/17/69 | JH | 9/10 TG 7 days 9/10 SAG 10 days OK | 6/28/69 | JH |
| / | NITROGEN " | 1 | 6/17/69 | JH | 105, 106 ppm | 7/10/69 | JH |
| / | FREE FORMALDEHYDE | 4 | 6/17/69 | JH | 26.5 mcg/ml | 6/30/69 | JH |
| / | MERCURY | | | | 61.5 mcg/ml | 7/1/69 | JH |
| / | ANIMAL TESTING | 11 | 8/25/69 | JH | See Protocol | | |
| / | GUINEA PIG SAFETY | | | | | | |
| / | MOUSE SAFETY | | | | | | |
| / | MONKEY SAFETY | | | | | | |
| / | MONKEY POTENCY | | | | | | |
| / | MANUFACTURING FINE | 6 | 6/17/69 | | | | |

Checked By Dan H. Williams Supervisor [Signature]

LABORATORY

0

Specimen: Stachylococcus Enterotoxoid 8"

Date: 10-16-69

Size: 5cc

Lot No. 96627

Code:

Area and Equipment Involved in Incident: Dr. W. H. H. H.

| Pectology Cells & Description | | | Amount Used |
|--|------------------|---------------------------------|-------------|
| 1. <u>Stachylococcus Enterotoxoid 8"</u> | <u>Condition</u> | <u>Labeled 974 Disposed 526</u> | <u>1520</u> |
| 2. <u>Stachylococcus Enterotoxoid 8"</u> | <u>Condition</u> | | |
| 3. <u>Stachylococcus Enterotoxoid 8"</u> | <u>Condition</u> | | |
| 4. <u>Stachylococcus Enterotoxoid 8"</u> | <u>Condition</u> | | |
| 5. <u>Stachylococcus Enterotoxoid 8"</u> | <u>Condition</u> | | |

Labeled by Hand

| Amount Labeled | Date | Amount to Finish | Date |
|----------------|-----------------|------------------|-----------------|
| <u>974</u> | <u>10-16-69</u> | <u>968</u> | <u>10-16-68</u> |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

Dr. W. H. H. H.
W. H. H. H.

PROTOCOL OF CONTROL TEST RESULTS
Staphylococcus Enterotoxoid B, Lot 96627

Tests Performed on Toxoid:

A. Sterility

- | | | |
|---------------|-----------------|------------------|
| 1. Final Bulk | Date on 6/13/69 | Date off 6/23/69 |
|---------------|-----------------|------------------|

1 ml of toxoid was inoculated into each of ten 400 ml bottles of Thioglycollate media. Incubated at 32°C.

Results: All 10 bottles satisfactory - observed 11 days.

- ## 2. Final Container

1 ml of toxoid from each of 10 final containers of toxoid was inoculated into 100 ml bottles of each of the following media.

Thioglycollate at 32°C. Date on 6/18/69 Date off 6/25/69

Results: All 10 bottles satisfactory - observed 7 days.

Sabouraud at 22°C. Date on 6/18/69 Date off 6/28/69

Results: All 10 bottles satisfactory - observed 10 days.

B. Mouse Safety

(Final Container)

Date on 8/26/69 Date off 9/2/69

Each of 4 mice, 20 - 22 grams, inoculated intraperitoneally with 0.5 ml of toxoid.

Results: All mice appeared normal at end of 7 day observation period. Three mice gained weight and one mouse lost 1 gram. Two of 4 control mice inoculated with saline lost 0.5 and 1 gram respectively. Necropsy showed all 4 test animals and all 4 control animals to be normal.

C. Guinea Pig Safety

(Final Container)

Date on 8/26/69 Date off 9/2/69

Each of 2 guinea pigs, 355 - 400 grams, inoculated parenterally with 5.0 ml of toxoid and each of 2 guinea pigs received 2.5 ml.

C. Guinea Pig Safety (continued)

Results: All 4 guinea pigs satisfactorily completed the 7-day test. All animals gained weight and appeared normal. At necropsy all 4 animals were normal.

D. Monkey Safety Test
(Final Container)

Date on 8/29/69 Date off 9/12/69

Each of four SEB-HA negative cynomolgus monkeys weighing between 2.36 and 3.2 kg. were injected subcutaneously with 5.0 ml of toxoid. The animals were observed for 14 days.

Results: All animals appeared normal from the 4th to the 14th days. Two animals vomited on Day 1. All four animals had poor or fair appetites through the 3rd day before becoming normal on the 4th day. No abnormal reaction was noted at the site of injection. However, delayed absorption of several days was noted for two animals.

E. Chemical Assays

- | | |
|---|--------------|
| 1. Nitrogen (Micro Kjeldahl Procedure) (Final Container) | 105.5 mcg/ml |
| 2. Free Formaldehyde (Final Container) | 26.5 mcg/ml |
| 3. Thimerosal (Final Container) | 61 mcg/ml Hg |

MONKEY DETOX TEST

Sample No. 1406D Vaccine Lot No. 96627 Product Staph. Enterotoxoid B
 Number & Species on Test 6 Cynos. - Charge No. -
 Date of last TB test 7/9/68 Date pre-injection bleeding 11/20/68
 Dates of 1.6 ml subcutaneous injections 1st 11/20 by H.R. 2nd None by
 Nitrogen: 311.5 mcg./ml.

Daily Observations

| Tag No. | Pre-Test SEB-HA | Observe 1st 16 Hr. | 11/21 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|--------------|-----------------|--------------------|-------|-----|-----|-----|------|-------|-------|-------|-------|-----|-----|-----|-----|-----|------|
| 13N | < 10 | Normal | 1 | 6 | 6 | 6 | 6,7 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 |
| 11N | 640 | Normal | 2 | - | - | - | 7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 |
| 19N | < 10 | Diarrhea* | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 20N | < 10 | Normal | 2 | - | - | - | - | 9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 |
| 22N | < 10 | Normal | 2 | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 18N | 80 | Normal | 2 | 6,7 | 6,7 | 6,7 | 6,7 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 |
| | | | | | | | | | | | | | | | | | |
| Observed by: | | | | | | | H.R. | | | | | | | | | | W.W. |

Test results Satisfactory by T.D.

Symptoms:

- 1 - Bloody stool
- 2 - Diarrhea
- 3 - Not eating
- 4 - Vomiting

- 5 - Inactive
- 6 - Edema of injection site
- 7 - Erythema of injection site
- 8 - Disorientation
- 9 - Necrosis
- = No symptoms

Responsible Operator Ted Dayhuff

*Non-specific.

Completion Date 12/4/68

STAPHYLOCOCCAL ENTEROTOXOID B -- Monkey Poteny Test

Sample No. 1406D Lot No. 96627
TAXIN: Lot No. 14-31R
Dilute 10 mg q.s. 10 ml Saline = 1.0 mg/ml by WD - LC
Inject 0.3 ml/kg I.V. for 300 mcg/kg challenge dose.
Dilute 1 ml of 1.0 mg/ml toxin + 9 ml Saline = 100 mcg/ml
Inject 0.1 ml/kg I.V. for 10 mcg/kg challenge dose.

| Monkey | | Weight | | | | Blood Data | | | | Challenge Data | | | | |
|--------|------|------------|---------|-------------|---------|------------|--------|-------------|--------|----------------|--------|---------|---------|-------|
| | | Pre-Chall. | | 14 Day Post | | Pre-Chall. | | 14 Day Post | | SEB-HA | mcg/ml | ml/Kg | ml/Dose | Date |
| No. | Shpt | Date | Wt (Kg) | Date | Wt (Kg) | Date | SEB-HA | Date | SEB-HA | mcg/ml | ml/Kg | ml/Dose | Date | Time |
| 100 | 267 | 9/2/69 | 1.83 | 9/16/69 | 1.30 | 9/2/69 | 40 | 9/16/69 | 80 | 1,000 | 0.3 | 0.55 | 9/2/69 | 10:50 |
| 101 | " | " | 2.30 | " | 2.16 | " | 320 | " | 80,000 | " | " | 0.69 | " | 10:52 |
| 102 | " | " | 1.86 | " | 1.85 | " | 640 | " | 40,000 | " | " | 0.56 | " | 10:54 |
| 103 | " | " | 2.12 | " | 1.62 | " | 320 | " | 10,000 | " | " | 0.64 | " | 10:55 |
| 104 | " | " | 1.79 | " | 1.59 | " | 320 | " | 5,120 | " | " | 0.54 | " | 10:58 |
| 105 | " | " | 1.63 | " | Dead | " | 640 | - | | " | " | 0.49 | " | 11:00 |
| 106 | " | " | 1.74 | " | 1.57 | " | 160 | 9/16/69 | 2,560 | 100 | 0.1 | 0.17 | " | 11:09 |
| 107 | " | " | 2.15 | " | 1.77 | " | 80 | 9/16/69 | 1,280 | " | " | 0.21 | " | 11:10 |
| 108 | " | " | 2.84 | " | 2.70 | " | 80 | " | 20,000 | " | " | 0.28 | " | 11:12 |
| 109 | " | " | 1.59 | " | 1.53 | " | 320 | " | 5,120 | " | " | 0.16 | " | 11:14 |
| 110 | " | " | 2.01 | " | 2.13 | " | 1,280 | " | 20,000 | " | " | 0.20 | " | 11:16 |
| 111 | " | " | 2.09 | " | 1.95 | " | 640 | " | 2,560 | " | " | 0.21 | " | 11:19 |
| 112 | " | " | 2.30 | " | Dead | " | <10 | - | | 1,000 | 0.3 | 0.69 | " | 11:02 |
| 113 | " | " | 3.23 | " | Dead | " | <10 | - | | " | " | 0.97 | " | 11:03 |
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| 117 | " | " | 1.71 | " | Dead | " | <10 | - | | " | " | 0.51 | " | 11:08 |
| 120 | " | " | 2.33 | " | 2.04 | " | <10 | 9/16/69 | <10 | 100 | 0.1 | 0.23 | " | 11:21 |
| 121 | " | " | 1.89 | " | Dead | " | <10 | - | | " | " | 0.19 | " | 11:22 |
| 122 | " | " | 2.10 | " | 2.00 | " | <10 | 9/16/69 | <10 | " | " | 0.21 | " | 11:24 |
| 123 | " | " | 1.90 | " | 1.74 | " | <10 | " | <10 | " | " | 0.19 | " | 11:25 |
| 124 | " | " | 2.58 | " | 2.41 | " | <10 | " | 80 | " | " | 0.26 | " | 11:26 |
| 126 | " | " | 2.15 | " | Dead | " | <10 | - | | " | " | 0.22 | " | 11:28 |

VACCINATES

51

CONTROLS

Responsible Operator Wm. Duling
Approved by W. G. Walter

STAPHYLOCOCCAL ENTEROTOXIN E Monkey Potency Test

| | by | WD - LC | Date |
|---|----|---------|---------|
| 1st Immunizing Dose = 0.5 ml Subcutaneously | | | 6/24/69 |
| 2nd Immunizing Dose = 0.5 ml Subcutaneously | | | 7/22/69 |

*10 ml blood sample taken by LC - JC Date 9/2/69

1 = Bloody stool
2 = Diarrhea
3 = Not eating
4 = Vomiting
5 = Inactive
6 = Edema of imm. site
7 = Erythema of imm. site
8 = Disorientation
- = No symptoms.

Responsible Operator Wm. Duling

MONKEY POTENCY ON STAPH. ENTEROTOXIN "B" TOXOID

A monkey potency test on Lot 96627 was started on June 24, 1969. Each of 12 monkeys was injected with 0.5 ml S.C. A second injection was given on July 22 and the monkeys challenged on September 2. Six vaccinates were challenged with 300 mcg/kilo. and six vaccinates challenged with 10 mcg/kilo. Twelve controls were used; six received a 300 mcg/kilo. challenge and six a 10 mcg/kilo. challenge.

I. 300 mcg/kilo. Challenged Vaccinated Monkeys

A. Results

Emesis, the first recorded symptom, was seen in five of the six monkeys. It occurred 6 times in Monkey 103 and one time in each of the other four monkeys. The first instance of vomition occurred within 50 minutes and as long as 100 minutes after inoculation.

Diarrhea was seen in all six monkeys. It occurred as soon as 3-3/4 hours postinoculation in Monkey 105 which subsequently died. The survivor had diarrhea initially between 43 and 61 postchallenge hours. The duration of diarrhea varied in the survivors from as little as a single instance in one monkey to repeated instances which occurred for a period as long as 7 days.

B. Summary

All six vaccinates became ill and one died.

II. 10 mcg/kilo. Challenged Vaccinated Monkeys

A. Results

Of the six vaccinated monkeys challenged, three showed no apparent symptoms. The other three each had 2 recorded instances of diarrhea on the first or second day before presenting a normal appearance.

B. Summary

Three of six animals remained normal. The other three had transient diarrhea of short duration. All animals survived.

III. 300 mcg/kilo. Challenged Control Monkeys

A. Results

The first symptom to appear in five of six control animals was emesis, which occurred within 41 minutes to 2 hours 15 minutes and was of short duration. Diarrhea of widely varying severity was evident in five of six animals. All six animals died within 28 to 42 hours following the challenge dose.

B. Summary

Before dying within 42 hours after challenge, six of six animals went through a period of emesis and/or diarrhea.

IV. 10 mcg/kilo. Challenged Control Monkeys

A. Results

Four of six animals exhibited diarrhea and/or emesis following challenge. One animal died without showing these symptoms. Two of the six animals died, one at 44 hours and the other at 55 hours after challenge.

B. Summary

Two of six animals died following challenge. Of the remaining four animals, one remained essentially normal in appearance and the other three presented a variety of symptoms.

| Monkey | Prechall. HA-Titer | Chall. Dose | Time following injection to first emesis/diarrhea | | Symptoms | | | |
|------------|-----------------------|-------------|--|---------|----------|----------|----------|-----------------|
| | | | Hours | Minutes | Emesis | Diarrhea | Lethargy | Appetite* Death |
| 100 | 40 | 300 mcg/kg. | 1 | 10 | x | x | - | 31% |
| 101 | 320 | 300 mcg/kg. | 1 | 25 | x | x | - | 39% |
| 102 | 640 | 300 mcg/kg. | 89 | 5 | - | x | - | 32% |
| 103 | 320 | 300 mcg/kg. | - | 50 | x | x | - | 57% |
| 104 | 320 | 300 mcg/kg. | 2 | 45 | x | x | - | 65% |
| 105 | 640 | 300 mcg/kg. | 1 | 15 | x | x | x | 0% |
| 106 | 160 | 10 mcg/kg. | - | - | - | - | - | 92% |
| 107 | 80 | 10 mcg/kg. | 1 | 50 | - | x | - | 95% |
| 108 | 80 | 10 mcg/kg. | - | - | - | - | - | 100% |
| 109 | 320 | 10 mcg/kg. | - | - | - | - | - | 100% |
| 110 | 1280 | 10 mcg/kg. | 2 | - | - | x | - | 95% |
| 111 | 1640 | 10 mcg/kg. | 4 | - | - | x | - | 95% |
| VACCINATES | | | | | | | | |
| 112 | <10 | 300 mcg/kg. | - | 45 | x | x | x | 4% |
| 113 | <10 | 300 mcg/kg. | 2 | 15 | x | x | x | 0 |
| 114 | <10 | 300 mcg/kg. | - | 40 | x | - | x | 0 |
| 115 | <10 | 300 mcg/kg. | - | 55 | x | x | x | 0 |
| 116 | <10 | 300 mcg/kg. | 1 | 10 | x | x | x | 9% |
| 117 | <10 | 300 mcg/kg. | 44 | 10 | - | x | x | 7% |
| 120 | <10 | 10 mcg/kg. | - | 55 | x | x | - | 62% |
| 121 | <10 | 10 mcg/kg. | - | 55 | x | x | x | 32% |
| 122 | <10 | 10 mcg/kg. | - | - | - | - | - | 100% |
| 123 | <10 | 10 mcg/kg. | 28 | 5 | - | x | - | 69% |
| 124 | <10 | 10 mcg/kg. | 23 | 5 | - | x | - | 73% |
| 126 | <10 | 10 mcg/kg. | - | - | - | - | x | 22% |
| CONTROLS | | | | | | | | |

*% = Eating observations/total appetite observations.

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 4237J8

MANUFACTURING PROCESS SUMMARY

In Process Toxoid Lot No. 4237J8 Processing Started 10/2/68

Toxin Used - Lot 14-31R

| | |
|--|------------------|
| | 31 x 100 mg/vial |
| | 18 x 50 mg/vial |

Resuspend in 2 Liters 0.15 M K phosphate buffer pH 7.5

Containing 0.8% Formalin Date 10/2/68

Detoxification Started 37°C Date 10/2/68

Removed to 5°C Date 11/1/68

Time at 37°C = 30 days

Dialysis: Time 60 hours

Dialyzing Fluid - 0.02M K phosphate buffer pH 7.5

Dialyzing Fluid Volume - 64 liters

Thimerosal added to 1:10,000

Final Dialyzed Toxoid Volume - 2,422 ml

Filtration - Millipore 293 mm Date 11/21/68

Prefilter

RA

AP 32 257

AA

AP32 257

HA

AP32 257

Rinse solution - Physiological saline with 1:10,000 Thimerosal

Filtration Time - 2 hours

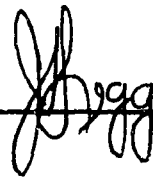
Post Filtration Volume - 2,874 g.

MANUFACTURING PROCESS SUMMARY - Page 2

Samples Removed for Adjuvant Experimentation - 125 ml

Final Bulk as Redispensed - No dilution or additives.

Approved by

_____

Effective: Nov. 1967

Approved: *[Signature]*

FILL ORDER

Product *Staphylococcus Enterotoxinoid B*

Final Container No. *Bulk As Dispensed* 4237JS

Dose _____ Size 100 cc Code _____ Max. — Cp. — Min. — Spg. _____

Expiration Date _____ Date Filled 6-17-69 Amount Filled 24X100cc 25.45 cc Total Fill Time _____

Area and Equipment Inspected and Satisfactory For Use Don E. Knudsen Date 6-17-69

| IBM No. | Packaging Codes and Descriptions | Amount Used |
|---------|----------------------------------|-------------|
| 30-0029 | 100 cc Type I Serum vial | 30 |
| 42 0014 | 20 mm 147 P Lfay Comb Seal | 60 |
| 30-0005 | Type I 5cc serum vial | 30 |

[illegible]

Checked By Det Graham Supervisor Hogey

May 1, 1969

Dr. McGann
U. S. Army Medical Unit
Fort Detrick
Frederick, Maryland 21701

Dear Dr. McGann:

Reference is made to our telephone conversation of yesterday concerning the difficulties which we have encountered in alum precipitating samples of the Staphylococcus Enterotoxoid B. Enclosed is a brief summary of the results obtained. It is, of course, distinctly possible that the presence of adjuvant even in view of this poor efficiency, could induce an acceptable immunological response. Your comments and suggestions will be greatly appreciated.

It is not unlikely that I may be out of the office frequently in the next several weeks. If you have any questions or comments by telephone, please ask for Mr. James S. Legg. He is thoroughly familiar with the work and all of the precipitations were performed by him or under his supervision.

With best regards.

Sincerely yours,

D. S. Mabry, Ph.D.
Director of Biologics

cc: Mr. James S. Legg

| <u>Precipitation</u> <u>No.</u> | <u>Method*</u> | <u>Supernatant</u> <u>2 hrs. @ R.T.</u> | <u>Supernatant</u> <u>Post-Centrifuge</u> | <u>Reconstituted</u> <u>Precipitate**</u> |
|------------------------------------|----------------|--|---|--|
| I | A | | 39 mcg/ml N ₂ pH 6.94 | 76 mcg/ml N ₂ 50 ml |
| II | B | | 91 mcg/ml N ₂ pH 8.4 | 31 mcg/ml N ₂ 50 ml |
| III | A | | 35 mcg/ml N ₂ pH 6.3 ca 60 ml | 36 mcg/ml N ₂ 50 ml |
| IV | B | | 33.6 mcg/ml N ₂ pH 8.14 ca 40 ml | 8.7 mcg/ml N ₂ 50 ml |
| V | C | | 23 mcg/ml N ₂ pH 7.5 63 ml | 36 mcg/ml N ₂ pH 7.8 25 ml |
| VI | C | 32 mcg/ml N ₂ (5 ml sample) | 30 mcg/ml N ₂ pH 7.6 41.5 ml | 65 mcg/ml N ₂ pH 7.8 25 ml |
| VII | D | 71 mcg/ml N ₂ (5 ml sample) | 33 mcg/ml N ₂ pH 7.6 18.4 ml | 54 mcg/ml N ₂ pH 7.45 25 ml |

*A = $K Al(SO_4)_2 \cdot 12H_2O + Na_2 CO_3 @ 37^\circ C. \rightarrow 16 \text{ mgm/ml } K Al(SO_4)_2 \cdot 12H_2O$ equivalent reaction in toxoid.

B = A precipitate was prepared using the ingredients at the same level as shown in A above. This preformed aluminum hydroxide was then added to the toxoid.

C = Same reagents as A. Level changed to 30 mgm/ml. Alkaline pH maintained at R.T.

D = $AlCl_3 + Na_3PO_4 @ R.T. \rightarrow 32 \text{ mgm/ml as } K Al(SO_4)_2 \cdot 12H_2O$ equivalent.

**Precipitates reconstituted to theoretical 100 mcg/ml N₂ based on toxoid 4237J8 assay 242 mcg/ml N₂.

PROTOCOL OF CONTROL TEST RESULTS
Staphylococcus Enterotoxoid B, Lot 4237J8

Tests Performed on Toxoid:

A. Sterility - Final Bottle, Small

10 ml toxoid from 1 small sample bottle was inoculated in the volume of 1.0 ml into each of ten 100-ml bottles of Thioglycollate media. Incubated at 32°C.

Date on 6/18/69 Date off 6/25/69

Results: All 10 bottles satisfactory - observed 7 days.

B. Chemical Assays

1. Nitrogen (Micro Kjeldahl Procedure)

Final bottle, small

264 mcg/ml

2. Free formaldehyde

Final bottle, small

46 mcg/ml

MONKEY DETOX TEST

Sample No. 431D Vaccine Lot No. 4237J8 Product Staph. Enterotoxoid B
 Number & Species on Test 6 Charge No. -
 Date of last TB test 7/9/68 Date pre-injection bleeding 11/4/68
 Dates of 1.6 ml subcutaneous injections 1st 11/4 by H.R. 2nd None by
 Nitrogen: 280.5 mcg./ml.

Daily Observations

| Tag No. | Pre-Test SEB-BA | Observe 1st 16 Hr. | 11/5 | | | | | | | | | | | 11/18 | | | |
|--------------|--------------------|-----------------------|--------------|------|------|---------|------|------|------|------|------|------|------|-------|------|------|------|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| 1N | < 10 | Normal | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6.7 |
| 2N | < 10 | Normal | - | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 5N | < 10 | Normal | 6.7 | 6.7 | 6.7 | 7 | 6.7 | 7 | 7 | 7 | 7 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 |
| 7N | < 10 | Normal | 6 | 6 | 6 | 6 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 |
| 9N | < 10 | Normal | 6.7 | 6.7 | 6.7 | 6.7 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 |
| 10N | < 10 | Normal | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Observed by: | | | J.E. | W.W. | W.W. | W.D. | W.D. | W.D. | J.E. | J.E. | W.W. | W.W. | W.W. | W.W. | W.W. | W.W. | J.E. |
| Test results | | | Satisfactory | | | by T.D. | | | | | | | | | | | |

Symptoms:

- 1 - Bloody stool
- 2 - Diarrhea
- 3 - Not eating
- 4 - Vomiting

- 5 - Inactive
- 6 - Edema of injection site
- 7 - Erythema of injection site
- 8 - Disorientation

- 9 - Necrosis
- - No symptoms

Responsible Operator Ted Dayhuff

Completion Date 11/18/68

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 4261J8

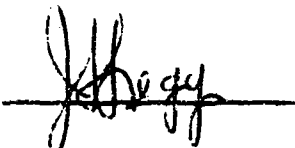
MANUFACTURING PROCESS SUMMARY

In Process Toxoid Lot No. 4261J8 Processing Started 10/16/68
Toxin Used - Lot 14-31R 31 x 100 mg/vial
 18 x 50 mg/vial
Resuspended in 2 Liters 0.15M K phosphate buffer pH 7.5
 Containing 0.8% Formalin Date 10/16/68
Detoxification Started 37°C Date 10/16/68
Removed to 5°C Date 11/15/68
 Time at 37°C = 30 days
Dialysis: Time 48 hours
 Dialyzing Fluid - 0.02M K phosphate buffer pH 7.5
 Dialyzing Fluid Volume - 64 Liters
 Thimerosal Added to 1:10,000
 Final Dialyzed Toxoid Volume - 2,640 ml
Filtration - Millipore 293 mm Date 12/30/68
 Prefilter
 RA
 AP32 257
 AA
 AP32 257
 HA
 AP32 257
 Rinse solution - Physiological Saline with 1:10,000
 Thimerosal
Filtration Time - 45 minutes

MANUFACTURING PROCESS SUMMARY - Page 2.

Post Filtration Volume - 3,078 g.

Final Bulk as Redispensed - No Dilution or Additives

Approved by 

4261 J8

FILL ORDER

FINAL GUN HS RE-SPENSED

Final Container No.

426/J8

Expiration Date _____ Date Filled 6-18-69 Amount Filled 30X5cc Total Fill Time 15 min

Area and Equipment Inspected and Satisfactory For Use *Dryden Station* Date *6-18-69*

| IBM No. | Packaging Codes and Descriptions | Amount Used |
|---------|----------------------------------|-------------|
| 30-0029 | 100cc Type I Serum Vial | 30 |
| 30-0033 | 5cc Type F Serum Vial | 30 |
| 42 0014 | 20mm 147 Pkay Comb. Set | 60 |

[illegible]

Checked By Jay K. Gustafson Supervisor

PROTOCOL OF CONTROL TEST RESULTS

Staphylococcus Enterotoxoid B, Lot 4261J8

Tests Performed on Toxoid:

A. Sterility - Final Bottle, Small

10 ml of toxoid from 1 small sample bottle was inoculated in the volume of 1.0 ml into each of ten 400-ml bottles of Thioglycollate media. Incubated @ 32°C.
Date on 6/19/69 Date off 6/26/69

Results: All 10 bottles satisfactory - observed 7 days.

B. Chemical Assays

1. Nitrogen (Micro Kjeldahl Procedure)
Final Bottle, Small 185.5 mcg/ml
2. Free Formaldehyde
Final Bottle, Small 44.85 mcg/ml

MONKEY DETOX TEST

Sample No. 1408D Vaccine Lot No. 4261J8 Product Staph. Enterotoxoid B
 Number & Species on Test Cynos. Charge No. -
 Date of last TB test 7/9/68 Date pre-injection bleeding 11/20/68
 Dates of 1.7 ml subcutaneous injections 1st 11/20 by H.R. 2nd None by
 Nitrogen: 326.5 mcg./ml

Daily Observations

| Tag No. | Pre-Test SEB-HA | Observe 1st 16 Hr. | 11/21 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|--------------|-----------------|--------------------|-------|---|-----|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 15N | < 10 | Normal | | | 7 | - | 6,7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6,7 |
| 24N | 1280 | Normal | | | 6,7 | 6,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 |
| 25N | 1280 | Normal | | | 6 | - | 6,7,9 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7,9 |
| 26N | < 10 | Normal | | | 6,7 | 6,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 | 6,7,9 |
| 45N | < 10 | Normal | | | 6,9 | 6,9 | 6,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 | 7,9 |
| 47N | < 10 | Normal | | | - | - | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | - |
| Observed by: | | | | | | | H.R. | | | | | | J.E. | J.E. | | | W.W. |

Test results Satisfactory by T.D.

Symptoms:

- 1 = Bloody stool
- 2 = Diarrhea
- 3 = Not eating
- 4 = Vomiting

5 = Inactive

6 = Edema of injection site

7 = Erythema of injection site

= Disorientation

9 = Necrosis

- = No symptoms

Responsible Operator Ted Dayhuff

Completion Date 12/4/68

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 4291K8

MANUFACTURING PROCESS SUMMARY

In Process Toxoid Lot No. 4291K8 Processing Started 11/6/68

Toxin Used - Lot 14-31R 33 x 100 mg/vial

14 x 50 mg/vial

Resuspended in 2 Liters 0.15M K phosphate buffer pH 7.5.

Containing 0.8% Formalin Date 11/6/68

Detoxification Started 37°C Date 11/6/68

Removed to 5°C Date 12/6/68

Time at 37°C = 30 days

Dialysis: Time 48 hours

Dialyzing Fluid - 0.02M K phosphate buffer pH 7.5

Dialyzing Fluid Volume - 64 liters

Thimerosal Added to 1:10,000

Final Dialyzed Toxoid Volume - 2,644 ml

Filtration - Millipore 293 mm Date 1/7/69

Prefilter

RA

AP32 257

AA

AP32 257

HA

AP32 257

Rinse solution - Physiological saline with 1:10,000
Thimerosal

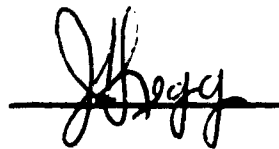
Filtration Time - 30 minutes.

Post Filtration Volume 3,071 g.

MANUFACTURING PROCESS SUMMARY - Page 2.

Final Bulk as Redispensed - No dilution or additives.

Approved by

A handwritten signature in black ink, appearing to be "J. H. G.", written over a horizontal line.

Approved: *[Signature]*

FILL ORDER

Dose _____ Size 100 cc Code _____ Max. - Cp. - Min. - Spg. _____

Expiration Date _____ Date Filled 6/15/69 Amount Filled 281046 Total Fill Time 15 min.

Area and Equipment Inspected and Satisfactory For Use Don W. Hutchison Date 6-18-69

| Sample No. | Type of Test | Volume | Date | By | Results | Date | Op. |
|------------|-------------------|--------|---------|----|-----------------|------|------------|
| 1 | STERILITY | 2.5ml | 6/18/64 | JH | 0/10 TG 7 drops | OK | 6/24/64 JH |
| 2 | NITROGEN | 5ml | 6/18/64 | JH | 200, 308 ppm | | 7/10/64 JH |
| 3 | FREE FORMALDEHYDE | 2.5ml | 6/18/64 | JH | 10.5 mg/ml | | 6/30/64 JH |

| | | |
|-------------------------------|---------|-------|
| Labeled by hand | 10/9/69 | |
| 29 x 100 cc & 25 x 5 cc vials | | 29 RD |

Checked By A. J. [Signature] Supervisor [Signature]

PROTOCOL OF CONTROL TEST RESULTS

Staphylococcus Enterotoxoid B, Lot 4291K8

Tests Performed on Toxoid:

A. Sterility - Final Bottle, Small

10 ml of toxoid from one small sample bottle was inoculated in the volume of 1.0 ml into each of ten 400-ml bottles of Thioglycollate media. Incubated @ 32°C.
Date on 6/19/69 Date off 6/26/69

Results: All 10 bottles satisfactory - observed 7 days.

B. Chemical Assays

1. Nitrogen (Micro Kjeldahl Procedure)
Final Bottle, Small 204 mcg/ml
2. Free Formaldehyde
Final Bottle, Small 40.5 mcg/ml

MONKEY DETOX TEST

Sample No. 1431D Vaccine Lot No. 4291K8 Product Staph. Enterotoxoid B
 Number & Species on Test 6 Charge No. -
 Date of last TB test 11/18/68 Date pre-injection bleeding 12/26/68
 Dates of 1.7 ml subcutaneous injections 1st 1/2 by T.D. 2nd None by -
 Nitrogen: 283 mcg./ml.

Daily Observations

| Tag No. | Pre-Test SEB-HA | Observe 1st 16 Hr. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 1/16 |
|--------------|-----------------|--------------------|--------------|------|------|---------|------|------|------|------|------|------|------|------|------|------|------|
| 15M | < 10 | Normal | 6 | 6 | 6 | 6.7 | 6.7 | 6 | 6 | 6 | 6 | 6 | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 |
| 2M | < 10 | Normal | 6 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| 3M | < 10 | Normal | 6.7 | 6 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| 4M | < 10 | Normal | 6 | 6.7 | 6 | 6.9 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| 5M | < 10 | Normal | 6 | 6 | 6 | 6.9 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| 6M | < 10 | Normal | 6 | 6 | 6 | 6.9 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| Observed by: | | | H.R. | H.R. | H.R. | W.W. | W.W. | H.R. | W.W. | W.W. | E.H. | E.H. | W.W. | W.W. | W.W. | W.W. | W.W. |
| Test results | | | Satisfactory | | | by T.D. | | | | | | | | | | | |

Symptoms:

- 1 = Bloody stool
- 2 = Diarrhea
- 3 = Not eating
- 4 = Vomiting

5 = Inactive

6 = Edema of injection site

7 = Erythema of injection site

8 = Disorientation

9 = Necrosis

- = No symptoms

Responsible Operator Ted Dayhuff

Completion Date 1/16/69

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 4292K8

MANUFACTURING PROCESS SUMMARY

In Process Toxoid Lot No. 4292K8 Processing Started 11/6/68

Toxin Used - Lot 14-31R

32 x 100 mg/vial

16 x 50 mg/vial

Resuspended in 2 Liters 0.15M K phosphate buffer pH 7.5.

Containing 0.8% formalin

Date 11/6/68

Detoxification Started 37°C

Date 11/6/68

Removed to 5°C

Date 12/6/68

Time at 37°C = 30 days

Dialysis: Time 48 hours

Dialyzing Fluid - 0.02M K phosphate buffer pH 7.5.

Dialyzing Fluid Volume - 64 Liters

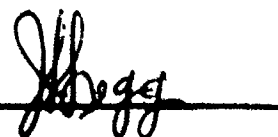
Thimerosal Added to 1:10,000.

Final Dialyzed Volume - 2,674 ml

Precipitate Removed for Experimentation - 75 ml

Final Bulk as Redispensed - No dilution or additives. Contains heavy precipitate.

Approved by



4292 188

FILL ORDER

~~Plant Container No.~~

429258

Expiration Date _____ Date Filled 6-18-79 Amount Filled 23.100 cc Total Fill Time 18 min.

Area and Equipment Inspected and Satisfactory For Use Don W. Huthman Date 6-18-69

[illegible]

Checked By Don H. Heston Supervisor

PROTOCOL OF CONTROL TEST RESULTS

Staphylococcus Enterotoxoid B, Lot 4292K8

Tests Performed on Toxoid:

A. Sterility - Final Bottle, Small

10 ml of toxoid from 1 small sample bottle was inoculated in the volume of 1.0 ml into each of ten 400-ml bottles of Thioglycollate media. Incubated @ 32°C.

Date on 6/19/69 Date off 6/26/69

Results: All 10 bottles satisfactory - observed 7 days.

B. Chemical Assays

1. Nitrogen (Micro Kjeldahl Procedure)
Final Bottle, Small 245.5 mcg/ml
2. Free Formaldehyde
Final Bottle, Small 49.9 mcg/ml

MONKEY DETOX TEST

Sample No. 1433D Vaccine Lot No. 4292K8 Product Staph. Enterotoxoid B
 Number & Species on Test 6 Cynos. - Charge No. -
 Date of last TB test 11/18/68 Date pre-injection bleeding 12/26/68
 Dates of 1.7 ml subcutaneous injections 1st 1/2 by W.W. 2nd None by -
 Nitrogen: 286 mcg./ml.

Daily Observations

| Tag No. | Pre-Test SEB-HA | Observe 1st 16 Hr. | 1/3 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|--------------|-----------------|--------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 8M | < 10 | Normal | 6 | 6,7 | 6 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 |
| 9M | < 10 | Normal | 6 | 6 | 6 | 6,9 | 6,9 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 |
| 10M | < 10 | Normal | 6 | 6 | 6 | 6 | 6 | 6,7 | 6,7 | 6 | 6 | 6 | 6 | 6,7 | 6,7 | 6,7 | 6,7 |
| 11M | < 10 | Normal | 6 | 6 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6 | 6 | 6 | 6,7 | 6,7 | 6,7 | 6,7 |
| 13M | < 10 | Normal | 6 | 6 | 6 | 6,9 | 6,9 | 6,9 | 6,9 | 6,9 | 6,9 | 6,9 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 |
| 14M | < 10 | Normal | 6,7 | 6,7 | 6,7 | 6,9 | 6,9 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 |
| Observed by: | | | H.R. | H.R. | H.R. | W.W. | W.W. | H.R. | W.W. | W.W. | W.W. | E.H. | E.H. | W.W. | W.W. | W.W. | W.W. |

Test results Satisfactory by T.D.

Symptoms:

- 1 = Bloody stool
- 2 = Diarrhea
- 3 = Not eating
- 4 = Vomiting

- 5 = Inactive
- 6 = Edema of injection site
- 7 = Erythema of injection site
- 8 = Disorientation

- 9 = Necrosis
- = No symptoms

Responsible Operator Ted Dayhuff

Completion Date 1/16/69

STAPHYLOCOCCUS ENTEROTOXOID B

Lot 87285

MANUFACTURING PROCESS SUMMARY

Toxoid Lot No. 87285 Processing Started 3/28/68

In Process Lot No. 4031C8

Toxin Used - Lot 14-31R 40 vials x 100 mg/vial

Resuspended in 2 Liters 0.15 M K phosphate Buffer pH 7.5

Containing 0.8% Formalin Date 3/28/68

Inactivated Started 37°C. Date 3/28/68

Removed to 5°C. Date 4/27/68

Time at 37°C. = 30 Days

Placed at Room Temperature Date 5/9/68

Placed at 37°C. Date 5/10/68

Removed to 5°C. Date 5/20/68

Additional Time at 37°C. = 10 Days

Total Time at 37°C. = 40 Days

Dialysis: Time 48 Hours

Dialyzing Fluid - 0.02M K phosphate Buffer pH 7.5

Dialyzing Fluid Volume - 64 Liters

Final Dialyzed Toxoid Volume - 2,559 g

Filtration - Millipore 293mm Date 6/7/68

Prefilter

RA

AP32 257

MANUFACTURING PROCESS SUMMARY - Page 2

Filtration - (continued)

AA

AP32 257

HA

AP32 257

Rinse Solution - Physiologic Saline

Filtration Time - ca 5 min.

Post Filtration Volume - 3,551 g

Final Bulk Prepared

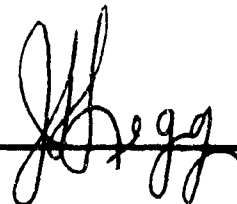
Date 6/14/68

Preservative Added - Thimerosal 1:10,000

Diluent - Physiologic Saline

Final Volume After Samples - 5,533 g

Approved by

A handwritten signature, likely "J. H. Egg", is written over a horizontal line.

SUMMARY OF FILLING AND LABELING OPERATION

Toxoid Lot No. 87285

Dosage Size 10

Volume 5 ml

Date Filled 6/25/68

Fill Volumes: Max. 6.5 ml Centerpoint 6.3 ml Min. 6.1 ml
with overfill

Filling Time 2 hours Amount Filled 871

Packaging Materials:

Type I 5cc Serum Vials
20 mm, 147 P Grey Combination Seals

Samples Taken:

| | | | |
|---------------------|-----------|-------------------|-----------|
| Sterility | 20 x 2 ml | Monkey Potency | 3 x 5 ml |
| Small Animal Safety | 5 x 5 ml | Chemical | 2 x 5 ml |
| Monkey Safety | 4 x 5 ml | Ft. Detrick | 20 x 5 ml |
| | | (Shipped 7/16/68) | |

Vials Rejected 41 (broken, cracked, etc.)

Labeled 776 vials Date Labeled 10/16/68

Additional Samples Taken:

Sterility Retest 20 x 5 ml
Mfg. File 6 x 5 ml

Finished Goods for Shipment 750 vials Date Shipped 10/22/68

Approved by 

PROTOCOL OF CONTROL TEST RESULTS

Staphylococcus Enterotoxoid B, Lot 87285

Tests Performed on Toxoid:

A. Sterility

1. Final Bulk Date on 6/14/68 Date off 6/25/68

1 ml of toxoid was inoculated into each of ten 400 ml bottles of Thioglycollate media. Incubated at 32°C.

Results: All 10 bottles Satisfactory - Observed 11 days.

- ## 2. Final Container

1 ml of toxoid from each of 20 final containers of toxoid was inoculated into 100 ml bottles of the following media.

Thioglycollate at 32°C. Date on 6/27/68 Date off 7/8/68

Results: 1 bottle contaminated with Gram positive rods on 8th day, 19 bottles Satisfactory - Observed 11 days.

Sabouraud at 22°C. Date on 6/27/68 Date off 7/8/68

Results: All 20 bottles Satisfactory - Observed 11 days.

Retest

An additional 20 final containers of toxoid were tested in Thioglycollate broth as per above.

Thioglycollate at 32°C. Date on 10/17/68 Date off 10/25/68

Results: All 20 bottles Satisfactory - Observed 8 days.

- ## B. Mouse Safety

(Final Container) Date on 7/16/68 Date off 7/23/68

Each of 4 mice, 18-20 grams, inoculated intraperitoneally with 0.5 ml of vaccine.

B. Mouse Safety (continued)

Results: All mice appeared normal at end of 7 day observation period and gained weight. Necropsy showed 1 mouse had nonspecific pneumonitis and the other three were normal.

C. Guinea Pig Safety
(Final Container)

Date on 7/1/68 Date off 7/8/68

Each of 2 guinea pigs, 300-400 gms, inoculated parenterally with 5.0 ml of toxoid and each of 2 guinea pigs received 2.5 ml.

Results: All 4 guinea pigs satisfactorily completed 7 day test and showed normal weight gains.

Necropsy indicated that the 2 pigs receiving doses of 5.0 ml and 2.5 ml subcutaneously to be normal; pigs receiving doses of 5.0 ml and 2.5 ml intraperitoneally showed fibrinous deposits on the surface of the viscera and trapped in the omentum, some mild peritonitis.

D. Monkey Safety Test
(Final Container)

Date on 7/1/68 Date off 7/15/68

Each of four SEB-HA negative cynomolgus monkeys weighing between 2.2 and 2.84 kg were injected subcutaneously with 5.0 ml of toxoid. The animals were observed for 14 days.

Results: All monkeys appeared normal throughout the observation period. Two showed weight gains and two remained at starting weight. No abnormal reaction was noted at the site of injection.

E. Chemical Assays

1. Nitrogen (Micro Kjeldahl Procedure)
(Final Container) 101.5 mcg/ml
2. Thimerosal (Final Container) 49 mcg/ml Hg
3. Formalin (Bulk) 0.016 %

MONKEY DETOX TEST

[illegible][illegible]

- Normal
D Hard, fibrinous deposit at injection site
S Ulceration at injection site

| | |
|----------------------|--------------------|
| Responsible Operator | <u>Ted Dayhoff</u> |
| Completion Date | <u>5/22/68</u> |

R. W. Cassen

Sample No. 6379 Toxoid Lot No. 87285 Product Staph. Enterotoxoid B
 Number & Species on Test 6 Cynos Charge No. -
 Date of last T3 test 4/12/68 Date pre-injection bleeding 5/22/68
 Dates of 15 ml subcutaneous injections 1st 5/22 by TD, 2nd - by -, 3rd - by -

| | | | | | | | |
|---------------------|--------------|----|----|--------|----|----|----|
| Observed by | WW | WW | WD | WD | JE | JE | JE |
| Date final bleeding | | | | | | | |
| Test Results | Satisfactory | | | by RWC | | | |
| Comments: | | | | | | | |

Responsible Operator Ted Dayhuff
Completion Date. 6/6/68

P. 15. Conf.

(Page 1 - Immunization)

STAPHYLOCOCCAL ENTEROTOXOID B
Monkey Potency Test

Sample No. 525D Lot No. 87285 Date on Test 7/1/68
Stage of Process Final Date of Challenge 9/9/68
Other Data _____

1st Immunizing Dose = 0.5 ml Subcutaneously by TD Date 7/1/68
2nd Immunizing Dose = 0.5 ml Subcutaneously by HR/JE Date 7/29/68

| Monkey | | TB Test | | Blood Data | | | Observations | | | | | Day 70* |
|--------|-------|----------|---------|------------|-----------|-------|--------------|--------|------|---------|---------|----------|
| No. | Shpt. | Wt. (Kg) | Date | Results | Screening | 0-Day | SEB-HA | SEB-HA | Date | 7/22/68 | 7/29/68 | Wt. (Kg) |
| 51D | 264 | 2.0 | 4/12/68 | Neq. | 5/1/68 | <10 | 7/1/68 | <10 | " | - | - | 1.93 |
| 40C | " | 2.84 | " | " | " | " | " | " | " | - | - | 2.75 |
| 48D | " | 2.2 | " | " | " | " | " | " | " | - | - | 2.35 |
| 47D | " | 2.6 | " | " | " | " | " | " | " | - | - | 2.56 |
| 46D | " | 2.0 | " | " | " | " | " | " | " | - | - | 2.27 |
| 41D | " | 2.6 | " | " | " | " | " | " | " | - | - | 2.78 |
| 45D | " | 2.6 | " | " | " | " | " | " | " | - | - | 2.78 |
| 44D | " | 2.3 | " | " | " | " | " | " | " | - | - | 2.84 |
| 54F | " | 3.4 | " | " | " | " | " | " | " | - | - | 3.38 |
| 66F | " | 2.5 | " | " | " | " | " | " | " | - | - | 2.73 |
| 62 | " | 3.6 | " | " | " | " | " | " | " | - | - | 3.58 |
| 50 | " | 2.6 | " | " | " | " | " | " | " | - | - | 2.92 |

* 10 ml blood sample taken by TD Date 9/9/68

Symptoms:

- 1 = Bloody stool
- 2 = Diarrhea
- = No symptoms
- 3 = Not eating
- 4 = Vomiting
- 5 = Inactive
- 6 = Edema of imm. site
- 7 = Erythema of imm. site
- 8 = Disorientation

Responsible Operator Ted Dayhoff

STAPHYLOCOCCAL ENTEROTOXOID B -- Monkey Potency Test

Sample No. 525D

Lot No. 87285

TOXIN: Lot No. 14-31R 10 mg q.s. 10 ml Saline = 1.0 mg/ml by TD
 Dilute 0.3 ml/Kg I.V. for 300 mcg/Kg challenge dose.

Dilute 1 ml of 1.0 mg/ml toxin + 9 ml Saline = 100 mcg/ml
 Inject 0.1 ml/Kg I.V. for 10 mcg/Kg. challenge dose.

| Monkey No. | Pre-Chall. | | | Weight | | | Blood Data | | | Challenge Data | | | | Time |
|------------|------------|--------|---------|---------|---------|------|-----------------|--------|---------|----------------|--------|-------|---------|---------|
| | Shpt. | Date | Wt (Kg) | Date | Wt (Kg) | Dead | Pre-Chall. Date | SEB-HA | Date | SEB-HA | mcg/ml | ml/Kg | ml/Dose | Date |
| 51 | 264 | 9/6/68 | 1.93 | 9/23/68 | 2.6 | Dead | 9/9/68 | 640 | 9/23/68 | 40,000 | 1000 | 0.3 | 0.58 | 9/11/68 |
| 40 | " | " | 2.75 | " | 2.3 | 2.6 | " | 320 | " | 80,000 | " | " | 0.83 | " |
| 48 | " | " | 2.35 | " | 2.3 | 2.3 | " | 320 | " | 80,000 | " | " | 0.71 | " |
| 47 | " | " | 2.56 | " | 2.5 | 2.5 | " | 640 | " | 80,000 | " | " | 0.77 | " |
| 46 | " | " | 2.27 | " | Dead | Dead | " | 160 | " | " | " | " | 0.68 | " |
| 43 | " | " | 2.78 | " | Dead | Dead | " | 20 | " | " | " | " | 0.83 | " |
| 45 | " | " | 2.78 | " | 2.68 | 2.6 | " | 80 | " | 1,280 | 100 | 0.1 | 0.28 | " |
| 44 | " | " | 2.84 | " | 2.60 | 2.60 | " | 320 | " | 10,000 | " | " | 0.28 | " |
| 54 | " | " | 3.38 | " | 3.20 | 3.20 | " | 640 | " | 10,000 | " | " | 0.34 | " |
| 66 | " | " | 2.73 | " | 2.70 | 2.70 | " | 2560 | " | 10,000 | " | " | 0.27 | " |
| 62 | " | " | 3.58 | " | 3.68 | 3.68 | " | 2560 | " | 20,000 | " | " | 0.36 | " |
| 50 | " | " | 2.92 | " | 3.0 | 3.0 | " | 5120 | " | 40,000 | " | " | 0.29 | " |
| 10 | " | " | 2.27 | " | Dead | Dead | " | <10 | " | " | 1000 | 0.3 | 0.66 | 8/17 |
| 39 | " | " | 2.30 | " | Dead | Dead | " | <10 | " | " | " | " | 0.69 | 8/40 |
| 37 | " | " | 2.56 | " | Dead | Dead | " | <10 | " | " | " | " | 0.76 | 8/42 |
| 21 | " | " | 2.22 | " | Dead | Dead | " | <10 | " | " | " | " | 0.66 | 8/45 |
| 16 | " | " | 2.61 | " | Dead | Dead | " | <10 | " | " | " | " | 0.78 | 8/47 |
| 24 | " | " | 2.61 | " | Dead | Dead | " | <10 | " | " | " | " | 0.78 | 8/49 |
| 18 | " | " | 2.50 | " | 2.60 | 2.60 | " | <10 | " | <10 | 100 | 0.1 | 0.25 | 9/08 |
| 20 | " | " | 3.41 | " | Dead | Dead | " | <10 | " | <10 | " | " | 0.34 | 9/10 |
| 22 | " | " | 2.73 | " | 2.70 | 2.70 | " | <10 | " | <10 | " | " | 0.27 | 9/12 |
| 26 | " | " | 2.50 | " | Dead | Dead | " | <10 | " | <10 | " | " | 0.25 | 9/14 |
| 33 | " | " | 2.67 | " | 2.60 | 2.60 | " | <10 | " | <10 | " | " | 0.27 | 9/16 |
| 65 | " | " | 3.52 | " | Dead | Dead | " | <10 | " | <10 | " | " | 0.35 | 9/18 |
| | | | | | | | | | | TD/JC TD/HR | | | | TD/HR |

Responsible Operator Ed Dayhoff
 Approved by R.W. Card

MONKEY POTENCY ON STAPH. ENTEROTOXIN "B" TOXOID

A monkey potency test on Lot 87285 was started on July 1, 1968. Each of 12 monkeys was injected with 0.5 ml S.C. A second injection was given on July 29 and the monkeys challenged on September 9. Six vaccinates were challenged with 300 mcg/Kilo. and six vaccinates challenged with 10 mcg/Kilo. Twelve controls were used; six received a 300 mcg/Kilo. chall. and six a 10 mcg/Kilo. challenge.

I. 300 mcg/Kilo. Chall. Vaccinated Monkeys

A. Results

The first symptom to appear following chall. was emesis, which occurred 80-90 minutes after chall. in the vaccinates as well as the controls. The number of times emesis occurred varied with each monkey, two of the six animals - 1 time, 1 - 6 times, 1 - 4 times, 1 - 3 times and 1 - 8 times. It should be noted that the one animal that showed emesis 8 times had the lowest SEB-HA titer.

The next symptom to appear was diarrhea and this appeared 24-27 hours following chall. and continued, in some instances, for the next 48 hours or until the animal died. Diarrhea was observed in all six vaccinates, but the number of times it occurred varied from animal to animal: one animal - only 3 times yet died, while another monkey exhibited loose stools 33 times in 48 hours and recovered.

B. Summary

All six vaccinates that received 300 mcg/Kilo. became ill. Three of the six animals died.

II. 10 mcg/Kilo. Chall. Vaccinated Monkeys

A. Results

Six vaccinated monkeys were challenged with 10 mcg/Kilo. of toxin. Of the six, only 1 animal showed any reaction to the chall. and emesis was the only symptom. This animal

11 A remained on feed and appeared normal 6 hours following chall. The remaining 5 test animals appeared normal throughout the test period. It is again interesting to note that the animal that became ill had the lowest HA titer of the six, suggesting that there may be a direct correlation between HA titer and the animal's ability to withstand chall.

B. Summary

Five out of 6 animals remained normal; one animal became ill, but recovered. 6/6 survived.

III. 300 mcg/Kilo. Chall. CONTROL Monkeys

A. Results

Six nonvaccinated monkeys were challenged with 300 mcg/Kilo. of toxin. The first symptom to appear was emesis and this occurred in all six animals. The number of times that emesis appeared in the animals varied considerably from animal to animal. Diarrhea occurred in all animals and, like the vaccinates, started about 24-28 hours following chall. All animals died within 60-72 hours following chall. dose.

B. Summary

6/6 animals went through a period of emesis-diarrhea. 6/6 died within 72 hours following chall.

IV. 10 mcg/Kilo. Chall. CONTROL Monkeys

A. Results

5/6 monkeys became ill, i.e., exhibiting diarrhea and/or emesis. One monkey remained normal throughout the observation period. 3/6 animals died within 96 hours after chall.

| Monkey | HA-Titer | Chall. Dose | Time Following Inj. to 1st Symptom | Symptoms | | | | Urethral |
|--------|----------|---------------|---------------------------------------|----------|------------|-----------|------|----------|
| | | | | Emesis | Diarrhea | Lethargic | N.E. | |
| 51 | 640 | 300 mcg/Kilo. | 128 min. | x | x | x | x | x |
| 40 | 320 | 300 mcg/Kilo. | 63 min. | x | x | - | x | - |
| 48 | 320 | 300 mcg/Kilo. | 87 min. | x | x | - | x | - |
| 47 | 640 | 300 mcg/Kilo. | 82 min. | x | x | - | x | - |
| 46 | 160 | 300 mcg/Kilo. | 62 min. | x | x | x | x | x |
| 43 | 20 | 300 mcg/Kilo. | 77 min. | x | x | x | x | x |
| 50 | 5120 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 62 | 2560 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 66 | 2560 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 54 | 640 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 44 | 320 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 45 | 80 | 10 mcg/Kilo. | 90 min. | x | - | - | - | - |
| 10 | <10 | 300 mcg/Kilo. | 71 min. | x | x | x | x | x |
| 16 | <10 | 300 mcg/Kilo. | 115 min. | x | x | x | x | x |
| 21 | <10 | 300 mcg/Kilo. | 76 min. | x | x | x | x | x |
| 24 | <10 | 300 mcg/Kilo. | 73 min. | x | x | x | x | x |
| 37 | <10 | 300 mcg/Kilo. | 100 min. | x | x | x | x | x |
| 39 | <10 | 300 mcg/Kilo. | 103 min. | x | x | x | x | x |
| 22 | <10 | 10 mcg/Kilo. | - | - | - | - | - | - |
| 20 | <10 | 10 mcg/Kilo. | 110 min. | x | x | x | x | x |
| 18 | <10 | 10 mcg/Kilo. | 66 min. | x | - | - | - | - |
| 26 | <10 | 10 mcg/Kilo. | 26 hrs. | - | x | x | x | x |
| 33 | <10 | 10 mcg/Kilo. | 78 min. | x | x (slight) | - | - | - |
| 65 | <10 | 10 mcg/Kilo. | 67 min. | x | x | x | x | x |